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# On the Symbiosis of Viruses and Microbes

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Soviet biological science has ascertained the decisive role of the outer medium in the development and variability of organisms. The great Soviet scholars, I. P. Pavlov and I. V. Michurin in different ways, while studying the various phenomena of nature, came to one and the same position on the unity of the organism and medium. Numerous scholars of our country with T. D. Lysenko at the head are successfully solving the questions of development heredity, variability, and formation of species in continuous harmony with conditions of life.

Productive use of this principle, which has enriched Soviet science with the greatest theoretical and practical achievements, requires a detailed study of organisms in close cooperation with the medium during various and changing conditions of this interaction. Offering special difficulties is a similar study of the different parasitizing organisms for which the inner medium of other organisms is, in certain periods of their existence, the outer medium and which do not breed in the outer natural medium. The viruses too belong to such parasitizing organisms. The viruses, being obligatory cellular parasites, in the opinion of the majority of investigators are deprived of the possibility to exist in an outer natural medium. It is quite natural that contemporary virology has paid much attention to a study of the

interaction of the viruses with the cells of macroorganisms. The question even of the interaction of viruses with the outer natural medium has been studied almost exclusively on the plane of the investigation of the effect on viruses of temperature, humidity, and other such factors. It is easy to see that such a study cannot give a proper idea of the interaction of viruses with the outer natural medium. This medium is never sterile; it is always in one or another degree populated with microbes, and the constant presence of microbes in this medium cannot be ignored during the study of the interaction of viruses with the natural medium.

In characterising the dialectic method, I. V. Stalin wrote: "...any phenomenon in any sphere of nature can be converted into an absurdity if it is examined out of connection with the surrounding conditions, in breaking away from them; and, vice versa, any phenomenon can be understood and substantiated if it is considered in its indissoluble content with surrounding phenomena and its dependency on the phenomena surrounding it" ("History of VKP (b). Short Course", p. 101; 1950.). This position of I. V. Stalin should be a guiding one in a study of the relationships and connections between the organism and the medium. Only in this case, if we shall study the interaction of viruses with the outer natural medium in close connection with those conditions in which the virus is found in this medium, we shall be able to understand the basic rules of this reciprocity and take into consideration its importance for the existence and propagation of the viruses.

One of the constantly existing conditions of the outer natural medium is the presence in it of microbes. An overwhelming majority of the viruses is inevitably found with them, not only when they are to be found in the outer medium but also during their direct passing over from one organism to another, since the tissues of the organism through which the viruses infiltrate are not sterile. Hence, it follows that during the study of the interaction of the viruses with the outer medium it is necessary that special attention be paid to their interaction with the microbes.

The capacity of the viruses for intracellular parasitism arose as a result of their many centuries of evolution, in the course of which the outer medium was altered, and the macroorganisms and viruses were altered as well as the microbes that populated and were in contact with the outer medium of the tissue of the macroorganisms.

It is difficult to suppose that many centuries of close contact between different living beings have proved insensible to them and have not led to any forms of their coexistence. It is natural, therefore, to suppose that among the viruses and microbes there may exist both the phenomena of symbiosis and the phenomena of antagonism so prevalent in nature.

The probability of such a hypothesis is confirmed further by the following considerations. It has long been known that microbes can adsorb the most diverse substances: dyes, colloid metals, etc. On the other hand, the capacity of viruses to be adsorbed by different substrates is known and used for development of methods for their purification. Finally, the capacity has long been known of killed microbes to adsorb viruses. All these facts make valid also the hypothesis of the adsorption of viruses by live microbes. Similar adsorption, in which closest contact occurs between them could in itself be the factor that has stipulated in the range of evolution the appearance of any forms of adaptation of viruses to microbes.

Thus, the study of the interaction of viruses from the outer natural medium requires a study of their interaction with the microbes.

Part of this great problem, the phenomenon of the symbiosis of microbes and viruses, was studied in our laboratory from 1933 to 1937 in collaboration with the late E. I. Vostrukhova, to the memory of whom this work is dedicated. Our investigations attracted the attention of other authors, and in subsequent years rather considerable literature has been compiled on this problem. Now when the position of the harmony of organism and medium has become a guiding principle of Soviet biology it is opportune to supply certain results of the study of this problem posed and studied by Soviet scientists.

We shall not set forth here in chronological order the data to be had in literature to the present time. It is more expedient for us to give an account of these materials in their logical development. Since the question of adsorption of viruses by microbes is quite essential to the study of the symbiosis between them and, together with it, has separate theoretical and practical significance, we shall give an account of it before the others.

## Adsorption of Viruses by Live Microbes

The adsorption of viruses by different substrates has repeatedly served as the subject of investigation of different authors, however, the question of the adsorption of viruses by living microbes has almost not been studied at all.

In our investigations (Zil'ber and Beliaeva, 1935) the adsorption was studied of the virus of variolar vaccine by the Torula kephir yeast. The choice of the yeast for experiments on the study of the symbiosis of viruses and microbes was stipulated by the fact that yeasts are widely distributed microbes often found in the air and on the skin of man. Furthermore, they are large, easy to culture, and well-studied microorganisms. The very same strain, Torula kephir, was the first strain with which experiments on symbiosis were set up, and since these experiments gave positive results we continued working with this strain further.

Experiments on the study of the adsorption were set up in the following way. To a suspension of yeast in physiological solution of sodium chloride or in bouillon the virus of variolar vaccine was added in the form of a centrifugate or filtrate of moist "soskoba" (scraping?) from calf or rabbit or of testicular rabbit virus. The mixture was left standing in a thermostat at 37° for 2 hours, and in part of the experiments it was left stand for 24 hours in a refrigerator at 4-6°. For control, the same amount of virus was added to bouillon and preserved with the same conditions. At the expiration of the time indicated the mixture was centrifuged, the deposit was washed twice with large amounts of physiological solution, and the content of virus was determined then in the super-charged fluid, the wash waters, and the deposit. To the super-charged fluid obtained fresh yeast was again added, and the experiment was repeated again. Determination of the virus was made by titration on the skin of rabbits or the cornea of pigs (Translator's note: probably guinea pigs).

In the overwhelming majority of experiments the washed deposit of yeasts contained the virus of variolar vaccine, and positive results were obtained at 1: 10 and 1: 100 dilution of the deposit. The first wash waters contained the virus, sometimes in considerable amount (up to a 1: 100) dilution). The second wash waters did not disclose the virus in a single experiment. The

super-charged fluid after first contact with the yeast did not always give an apparent reduction of titer, especially if it was high, but after a second contact the titer was reduced from ten to a thousand times.

In experiments in which the yeasts were added to the virus, the filtrate of which had a low titer (1:100), the virus could not be detected in the super-charged fluid after first contact. These experiments revealed to us that living yeast can adsorb the virus from variolar vaccine.

Turevich and Ianushevich (1937) studied this question in more detail in our laboratory. They took into account in their experiments the pH of the medium in which the adsorption of the virus by the yeast was studied and the yeast was precipitated and washed (three to five times) during 3000 revolutions of the centrifuge in the course of 5 minutes.

Centrifugation was carried out a short enough time to avoid even an insignificant deposit, together with the yeast, of the variolar virus too. Suitable experiments showed that with the use of moist scrapings containing a vast amount of elementary bodies the latter are deposited, even if in an insignificant amount, during centrifugation for a period of 10 or more minutes. Experiments set up during the maintenance of all these precautions with filtrates of moist scraping revealed that yeast after contact with the variolar virus in the refrigerator for a period of 24-40 hours and after washing three times, contain this virus and provoke in animals typical variolar disease.

Adsorption of the virus in the most considerable amount was noted in the acid zone at pH = 5.4 - 6.2. At pH = 7.1 - 8.2 it was less sharply expressed and was not observed in all cases. Attempts to study the adsorption of the virus by microscopic examination of smears did not give Turevich convincing results. At the present time by means of electron optics (microscope?) this question can be investigated in great detail.

The question of the adsorption of the viruses by live microbes was studied also by Zeitlank (1950) on types of virus of infectious ectromelia. Filtrates of this virus were blended through a Berkefeld V or N candle filter with suspensions of

various microbes, the absence of the pathogenicity of which for mice was verified in preliminary experiments. The mixture was let stand in a thermostat 1.5 to 3 hours, then in the refrigerator for 20 hours, after which the fluid with microbes not yet precipitated was removed, and the microbe deposit was washed with repeated centrifugation. For control, the same amount of virus was added to the buffer mixture not containing microbes, and with this mixture the same handling was carried out as in the main experiment. The content of virus was determined by inoculation of mice 1) by filtrate, 2) by a mixture of filtrate and buffer, 3) by the last wash fluid of microbes, 4) by the last wash fluid of the control, 5) by the deposit of microbes washed from the filtrate, and 6) by the control precipitate. The following cultures of microbes were tested by the author: the yellow Sarcina, the white and the yellow Staphylococcus, B. mycoides, and various species of yeast (kephir, koumiss, the rose-colored, the thermophilic, and others). Adsorption was studied in relation to the pH of the medium, the temperature, the consistency of the microbe suspension, the time of contact of the virus with microbes, and so forth.

All these tests disclosed a definite capacity of the virus of ectromelia to be adsorbed by microbes and revealed, together with this, that the different microbes possess an entirely different capacity to adsorb the virus. Thus, for example, the staphylococci and sarcina in all 35 experiments disclosed this capacity, whereas the rose-colored yeast adsorbed the virus only in one out of 14 experiments, but in the koumiss yeast this capacity was not generally observed. In those experiments in which the phenomenon of adsorption was observed the washed microbes contained virus, but the last wash water and the controls did not contain virus. It should be noted that in certain cases the virus was adsorbed by microbes almost wholly, as the experiments revealed this in which the deposit was titrated for content of virus.

In the experiments of Zeitlenk, the same as in the experiments of Turevich and Iamushevich, the importance of the pH of the medium for adsorption was noted. As a rule, the adsorption proceeded considerably more fully during a slightly acid reaction, with the pH equal to 6.0-6.8. In conditions of a slightly alkaline medium (pH = 7.4-8.4) the number of positive results was considerably reduced. The substitution of a buffer solution of horse serum

did not inhibit adsorption. The virus in the adsorbed state proved more durable both to preservation in the conditions of the thermostat and refrigerator and to heating at 55° C.

It is interesting to note that the strain of kephir yeast which adsorbed well the virus of variolar vaccine in our experiments and the experiments of Turevich adsorbed the virus of ectromelia poorly in the experiments of Zeitlenk. Thus, in the phenomenon of adsorption both the properties of the microbe and the properties of the virus play a role.

All these data have left no doubt of the fact that at least certain viruses are adsorbed by microbes in the conditions of the experiment. Does this process take place in natural conditions? Pal'kovich and Ianushevich (1937) have devoted special investigation to this question.

They sowed fresh variolar calf lymph in 1: 10 dilution on blood agar poured into a Petri dish. After 24 hours of standing in a thermostat all species of the colonies cultured were removed from the dish to bouillon. The bouillon cultures were introduced intra-cutaneously into rabbits, which were killed with a blow on the 4th-5th day with the typical reactions present. The papules on the skin that were separated off from these rabbits were readily cut into from the inner side, and a small amount of exudate was collected, diluted with sterile physiological solution, and investigated for the presence of the virus of the vaccine after verification of the sterility. The presence of the virus was established by inoculation into the cornea of rabbit or into the chorio-allantoid membrane of chick embryo, with subsequent disclosure of the typical reaction and of the elementary bodies.

During the investigation of 90 strains of different microbes isolated from 30 series of calf lymph it was determined that out of 39 strains of white staphylococcus 34 were bearers of the virus of the variola vaccine, out of 14 strains of yellow sarcina ten strains were bearers of the virus, out of 15 strains of yellow staphylococcus six strains were bearers of the virus, and out of 22 strains of diphtheroid bacillus not one contained virus.

Thus, these experiments showed that the virus of variola vaccine can be adsorbed by certain microbes and, in natural conditions, adsorption here too bearing a selective character.

All these investigations of Soviet authors demonstrate with certainty the possibility of adsorption at least of certain viruses by microbes both in experimental and in natural conditions. Foreign authors until recently paid no attention to this question. Dujaric de la Riviere (1930) observed that staphylococcus isolated from variolar calf lymph provokes at inoculation into the skin of rabbit variolar pustule, and at inoculation keratitis on the cornea of (guinea?) pig. But the role of the staphylococcus in the reaction provoked by variolar vaccine interested him mainly.

In connection with our works, Amies (1936) studied the adsorption of the virus of the vaccine by the Torula kephir yeast and by white staphylococcus and did not observe the adsorption of the virus by these microbes. In the overwhelming majority of cases Amies used in his experiments a washed suspension of elementary bodies, in the preparation of which their adsorption capacity was possibly changed. Moreover, he did not take into account the pH of the medium in which the adsorption occurs. It is surprising that Amies did not observe the precipitation of the elementary bodies during their centrifugation for a period of 30 minutes at 3500 revolutions per minute. In the above-mentioned experiments of Traub the precipitation, as indicated, occurred at a far earlier period, and Amies should have been able to detect the virus in the yeast and staphylococcus precipitates if not in an adsorbed state, then as a result of the settling during centrifugation. However, Amies reports no such data. It is entirely likely that Amies' negative results were stipulated by the use of washed elementary bodies and the unfavorable hydrogen figure of the medium in which the experiments of adsorption were conducted.

In connection with our data cited above, as also in connection with observations that established the frequent occurrence in the outer medium of the virus of poliomyelitis, foreign investigators too have occupied themselves in recent years with the question of the possibility of adsorption and the carrier ability in general of viruses by microbes.

In our works it has been pointed out that the survival of viruses in the outer medium is possibly stipulated by their symbiosis with microbes. This opinion too is placed at the base of the works of foreign investigators. Kling and his collaborators, Olin, Fahraeus and Norlin, (1942) studied the presence of the virus

of poliomyelitis in sewer waters of one of the Stockholm districts. The number of virus detected was so considerable that it was necessary to assume either the possibility of the carrying of the virus by all the residents of this district (about 100,000 persons) or its multiplication in the sewer waters. The authors expressed the hypothesis that this multiplication occurs in symbiosis with protozoa of the genus Bodo.

Evans and Osterud (1946) tried to ascertain whether the virus of poliomyelitis propagates in cultures with protozoa (the Bodo genus, Monas, and several others) by adding to cultures grown in the most favorable conditions a suspension of spinal cord containing the virus. After incubation, for a period of 5-14 days, readings were made. The fluid of different generations, treated with ether and centrifuged for riddance of bacteria, was introduced into the brain of monkeys. The experiments disclosed no propagation of virus in the protozoa cultures. The authors did not study the phenomenon of the adsorption of the virus by the microorganisms.

The experiments of other authors have disclosed, however, a certain possibility of adsorption and the ability of protozoa and bacteria to carry the virus of poliomyelitis.

Toomey and coworkers, Takacs and Schaeffer, (1948) studied the possibility of the ability of Amoeba proteus to carry the virus of poliomyelitis. The authors cultured the amoebas in water to which bacteria, killed by boiling, had been added. After contact with the virus of poliomyelitis the amoebas were repeatedly washed (up to 11 times) with water and were then introduced into the brain of mice. Experiments revealed that amoebas can trap the virus of poliomyelitis or adsorb it securely on their surface. Washed with 50 cc. of sterile water from five to 11 times, they still infected mice, producing the typical disease. The authors note that the virus was not detected in the fifth wash water, but was detected in the 11th, and they explain this by the fact that only prolonged washing destroyed the membrane of the amoeba and admitted the virus to the surrounding fluid. Microscopic investigation actually disclosed considerable demolition of the amoebas after the 11th washing. The virus was not discovered in the amoebas 3, 4, and 6 days after contact.

Recently Young, Felsenfeld, and Byrd (1949) (here printed

as Heung, in the bibliography as Young) disclosed the possibility of the virus of poliomyelitis (the Lansing strain) to be adsorbed by E. hystolytica, as well as by the intestinal bacillus and by a variant of the hay bacillus. It is interesting to note that the authors observed the great immunity of the virus adsorbed by the amebas and bacteria to the temperature of the thermostat. The amebas contained the adsorbed virus after 48 hours of incubation of the cultures in the thermostat. No propagation of the virus was observed by the authors.

The aggregate of all of the above-mentioned facts leaves no doubt that the viruses are adsorbed by microbes and protozoa in experimental and in natural conditions.

This adsorption is not the usual physico-chemical process although it proceeds during definite physico-chemical conditions. The adsorption of the viruses by the microbes bears a selective character stipulated by the biological and not the physico-chemical peculiarities that have a hand in the reaction of the microorganism. One and the same virus is adsorbed to an entirely different degree, or is not adsorbed at all, by the various microbes in one and the same conditions. On the other hand, different viruses can be adsorbed by one and the same microorganism. All this permits thinking that the phenomenon of adsorption of viruses by microbes is a biological phenomenon connected with the adaptation of the virus to the outer medium.

## Symbiotic Cultures

The above-mentioned considerations have given occasion to suppose the possibility not only of the adsorption of viruses by microbes but also their multiplication in the microbe cells. Suitable experiments were set up by us and later on by other investigators with viruses of variolar vaccine, fowl variola, measles, herpes, rabies, encephalomyelitis of horses, swine plague, foot and mouth disease. The circumstance that the rickettsiae are distinguished from the viruses by the conditions of their culture has given occasion to study in symbiotic cultures the virus of exanthematous typhus. The symbiotic cultures of the virus of variola with different species of yeast were studied in greatest detail

## The Virus of Variolar Vaccine

In 1932 we reported (Zil'ber and Vostrukhova - translator's note: the latter is spelled Vostroukhova in the bibliography - 1932, 1933) on the possibility of growing the virus of variolar vaccine for a prolonged period in a bouillon culture of yeast. The filtrate of damp "zoskoba" (?scraping) was added to a 2-3-hour culture of Torula kephir yeast, and this culture was let stand in the thermostat at 37° C. Resowings were made every three days in a test tube with ordinary meat-peptone bouillon. In control test tubes the virus was added to the bouillon without yeast, and sowings were made at the same time. The content of virus in the test tubes was studied by intracornsal inoculation of pigs, as well as by intracutaneous and intratesticular inoculation of rabbits. In these experiments we used the Torula kephir strain.

Symbiotic cultures of the variola virus, which can be transferred ten times with preservation of virulency, were obtained by the above-mentioned method. One of the cultures was transferred over more than one and a half years and still kept its activity after the 120th resowing. A study of these cultures had revealed that they have a number of peculiarities that distinguish the virus in these cultures from the ordinary virus of the vaccine. One of these peculiarities consisted of the fact that the cultures did not take at cutaneous inoculation, but took well at intracutaneous introduction, as well as at intratesticular and intracerebral inoculation of rabbits and pigs, provoking typical alterations and the accumulation of elementary bodies (translator's note: same as inclusion bodies) in the tissues. The cultures took well also at inoculation into the cornea of animals. The lack of the capacity in symbiotic cultures to take at cutaneous inoculation was stipulated possibly by the close connection of the virus with the yeast cell. Experiments in which such inoculation was successful if the cultures were carefully pulverized beforehand in a mortar with sand served as proof of this hypothesis. The virulence of symbiotic cultures was in the first ten generations rather high. Thus, for example, strain 662 in the 39th generation took at dilution of 1: 100,000, strain 305 took in the 29th generation at dilution of 1: 50,000, etc.

The greatest dilution at which inoculation was observed to take was that of 1: 500,000. However, far from all strains had

similar virulency, and it did not remain constant. After several "decades" (tens) of generations the virulency usually dropped rather considerably. A special investigation made together with V. D. Timakov showed that not every yeast cell is a virus bearer. A person is easily convinced of this at sowing a symbiotic culture on agar and at studying the virus content of each colony. At selecting colonies that contain virus and at resowing them on bouillon it was possible to increase considerably the virulency of the symbiotic culture and to maintain it by this method at a high level.

In all cases at successful inoculation the culture always succeeded in disclosing elementary (inclusion) bodies in the tissues. But all attempts to disclose elementary bodies in the cultures produced no results. A study of the antigenic properties of symbiotic cultures disclosed their capacity to cause immunity and the formation of antibodies to the variolar virus a certain time after inoculation. However, the intensity of this process was not very sharply expressed. Thus, for example, the sera of rabbits immunized by symbiotic cultures completely neutralized 10 infectious doses of variolar calf lymph neutralisation of 20 - 100 doses was not constant. Pigs and rabbits immunized by cultures proved immune to subsequent inoculation by 10 infectious doses of variolar calf lymph, but this immunity was relatively brief and was not observed in all cases. Thus, the study of the antigenic properties of symbiotic cultures disclosed the presence in them of the variolar virus, but with somewhat altered properties.

A histological study of the skin of a rabbit inoculated with symbiotic cultures disclosed typical alterations and Guarnieri bodies (Khurgina, 1935).

It should be pointed out that the symbiotic cultures obtained by us of variolar virus were turned over to three institutes: to the L. A. Tarasevich Control (Institute), to the TsIUM, and to the Kharkov Mechnikov (Metchnikeff) Institute. Investigation of these cultures, both in the generations obtained by us and in subsequent ones, disclosed the presence in them of the variolar virus (Gil'gut, 1935; Korol'kova, 1936; Khasterich and Shur, 1935).

The aggregate of all these data have permitted us to make the conclusion that the virus of variola - vaccine can be propagated

in symbiotic cultures with certain yeasts and such cultures can be preserved for a long time in the laboratory. It is entirely natural that adaptation to yeast cells causes an alteration of the properties of a virus. This alteration is not, however, stable, and the virus from a yeast culture passed through animal tissues quickly returns to its initial properties.

Our investigations on symbiotic cultures of the virus of variola were verified and confirmed by a number of authors.

Gil'gut (1935) studied the symbiotic cultures of 16 strains of variolar vaccine with kephir yeast. In experiments from six of these strains symbiotic cultures showed quick weakening in the first generations and in all the rest the virus was detected in cultures of the 8th, 9th, 17th, 19th, 20th, and 23d generations. Also successful were certain of the author's experiments at cultivation of the virus in symbiotic cultures with staphylococcus and sarcina. Determination of the presence of the virus in the cultures was done by inoculation into the cornea, elementary bodies always being discovered in positive cases. Cutaneous inoculation of rabbits with symbiotic cultures produced no results, as was also the case in control experiments. At intracutaneous inoculation of yeast cultures into rabbits the authors observed only slight reactions and not once observed generalization. One of our yeast cultures of the virus of variola, brought by us in the laboratory to the 74th generation, was sent to Dr. Gil'gut, and she still detected the virus in this culture after 13 further generations (up to the 87th inclusively). The immunity test in seven rabbits inoculated with symbiotic cultures by smearing on their skins undiluted calf lymph gave negative results. The author, however, indicates that these results were stipulated by negligible reaction or by its absence at inoculation of the cultures.

Korol'kova (1935) studied three strains of symbiotic cultures of the virus of the variolar vaccine, two of which were obtained from our laboratory (4th and 8th generations) and one was obtained by sowing in kephir yeast the virus of the neurovaccine. The first two proved still active in the 34th and 20th generations, the latter in the 26th generation. The activity was controlled by inoculation into pig cornea and into the skin of rabbit, and the specificity in a considerable portion of the experiments was confirmed by the

discovery of elementary bodies. The author notes that the intracuticular inoculations of rabbits "were attended by the formation of pockmarks over the whole surface of the integument, reaction usually having been lacking at the place of the introduction of the needle and the pockmarks having appeared at a distance of 2-3 cm. from the place of injection". Inoculations of cultures into heifers were unsuccessful.

Positive results of cultivating the virus of variola in symbiosis with the yeast were also obtained by Isabolinskii, Levtsov, and Cherniak (1935). The activity of the cultures was maintained in their experiments up to the 17th-20th generations. Attempts to cultivate the virus on sarcinas produced no results. Yeast cultures of the virus kept their activity at resowing on agar, as well as at preservation for a prolonged period at room temperature.

Timakov (1936) cultivated the virus of variolar vaccine in symbiosis with kephir yeast, staphylococci, and sarcina. Cultures with yeast kept the virus active to the 24th generation, the same as certain cultures with staphylococci. Cultures with sarcinas were non-virulent after the 13th to the 21st generations.

The author not only studied the presence of virus in the cultures, but also determined its titer in many resowings. The titer of the virus proved sufficiently high, and certain cultures of distant resowings (15 - 17) were active in a dilution up to 1: 25,000. The cultures took for the rabbit only at intracutaneous introduction, and the smearing of cultures onto the skin gave no positive results. In these experiments there was also noted the capacity of yeast cultures to produce in rabbits a generalized process. By selection the author succeeded in considerably boosting the virulency of the cultures obtained by him. The kephir yeast both in his works and in the works of Gil'gut and Korol'kova was entirely apathogenic.

Tul'chinskaia (1936) studied in detail the question of the culture of the variola of fowls in symbiosis with kephir yeast. The virus in the yeast cultures was detected up to the 14th generation, whereas in control resowings in bouillon without the yeast it was possible to discover the virus no further than in the 3d-4th resowing. In skin affections observed at introduction of cultures elementary bodies were found. In certain cases at intra-

duction into the skin of a yeast culture (10th generation) a generalized process and the appearance of pockmarks on the mucosa of the mouth, eyelids, etc. were observed. The author observed weakening of the virulency from the 16th to the 18th generations and the disappearance of it from the 35th to the 44th generations. Yeast cultures of chicken pox in the 32th generation and of pigeon pox in the 16th generation provoked, moreover, distinct reaction and subsequent immunity to the natural virus. Immunity was verified in chickens by the smearing of natural chicken virus on the scarified skin of the comb and wattle and by implantation from sick chickens to immunized, and in pigeons by smearing pigeon virus onto the scarified follicles of the breast, and in both cases 45 days and 5-6 months (? respectively) after the initial affection. All of the chickens so tested and 19 of the pigeons did not fall sick from the variola, while there was sickness of one and all of the controls. An analogous experiment with yeast cultures of 20th to 21st generations gave negative results, and in the birds made ill again after infection with yeast cultures no immunity was discovered to subsequent introduction of natural virus. Tul'chinskaia noted a further interesting circumstance. She added healthy fowl to fowl that had been diseased by yeast cultures, provoking in them distinct disease. These healthy birds that had been in close contact with those that were inoculated did not fall ill, however, which permits supposing the modification of the virus in the symbiotic cultures and loss by it of contagiousness.

Bulanov and Rinkhovskii (1936) tried to culture the virus of variola, the diphtheria of fowl, in symbiosis with brewers' yeast and sarcinas. The authors tested only the second generation of their cultures, inoculating it into two chickens by smearing on scarified comb and wattle. At obtaining negative results, the authors did not continue their experiments. Negative results were also obtained by Amies (1935), who in his main experiments used washed elementary bodies of variolar vaccine, as well as Voet (1935) in some experiments with neurovaccine, and Arbeit (1935) in experiments with vaccine and exanthematous typhus.

All the above-mentioned data leave no doubt of the fact that the virus of variolar vaccine can keep its activity for a long time in symbiotic cultures with yeast and certain bacteria. All investigators who followed our method and did not abandon their works after unsuccessful experiments with the first cultures of

microbes and strains of virus that fell into their hands received positive results.

## Other Viruses

Besides the symbiotic cultures of the virus of variola, there were studied symbiotic cultures of other viruses also. Smirnov and coworkers (1935) cultivated the virus of measles in symbiosis with kephir, bread, and brewers' yeast. The blood of measle patients, taken at onset, was diluted with Tyrode's solution in the proportion 1: 7 - 1: 10, and 3-5 drops of this blood were sown in a test tube with freshly sown yeast. Resowings were made every 6-7 days, and detection of the virus in the cultures was made by inoculation of rabbits and in other experiments. The control rabbits were inoculated with the blood of patients, taken at onset, as well as by the initial yeast culture. Parallel with these experiments there were set up experiments of the inoculation of rabbits with measle virus cultures on River's medium. Judgment was made of the specific reaction of the rabbits by the rise of temperature 5-14 days after infection, by the appearance of the catarrhal phenomena of the conjunctivae and of the mucosa of the nose, by leukocytosis in the first 24-48 hours after infection, and subsequent leukemia and alteration of the hemogram. The specific pathogenicity was disclosed in other experiments too. A comparison of all the data obtained permitted the authors to conclude that the measles virus is preserved in yeast cultures for a period of 35 or more generations.

Unfortunately the authors did not learn of our instruction on the necessity to use for cutaneous inoculation pulverized cultures. It is entirely likely that had they so done their results would have been even more demonstrative. Afanas'eva and Shapiro (1936) also studied the possibility of cultivation of measles virus in symbiosis with kephir yeast. They came to the conclusion that such cultivation does not turn out well in all cases and symbiotic cultures possess weak pathogenicity for rabbits. Degviz (1927) observed prolonged survival of measles virus in cultures with different cocci.

Izabolinskii, Levtsov, and Cherniak (1935) reported on their

obtaining symbiotic cultures of rabies virus with kephir yeast. The data cited by them, however, are unconvincing. The loss (or killing?) of rabbits on which testing of the cultures was carried out was observed on the second day when there were phenomena of paralysis. No bacteriological controls were set up. Insufficient passages were made. No histological investigations were made of the brain.

Rosengol'ts and Karnaukhova (1934) cultivated the virus of rabies on Micr. lysodeicticus. The cultures were tested after 5-12 days' growth by inoculation of rabbits and pigs subdurally and of mice into the muscles of the femur.

One of the cultures (the 10th generation) was inoculated into 27 mice, 13 rabbits, and 4 pigs. Dying from rabies with the confirmation of further passages were 11 mice, 3 rabbits, and two pigs. Incubation in the majority of cases was from 25-60 days, although the three-day was also observed. The authors reported likewise on certain successful experiments at cultivating the virus of rabies with kephir yeast. In cultures of micrococcus the authors also grew the virus of street rabies. A dog was inoculated with the 6th generation; it died with the phenomena of paralysis 7 days after inoculation. Its brain was passed through rabbits, in 18 out of the 26 animals paralysis having been observed. In two rabbits inoculated intramuscularly with the brain of the dog, inclusions were observed similar to Negri bodies, and in subsequent passages into animals these formations were not observed.

Akker and Florinskii (1935) obtained no results in attempts to cultivate in symbiotic cultures the virus of rabies. In their experiments with kephir and brewers' yeast there were observed not only propagation but also survival of the virus. Moreover, the yeasts at intracerebral inoculation proved pathogenic. Experiments with sarcina were successful.

Palavandov, Serebriansia, and Pugach (1936) cultivated the virus of rabies in mixed culture with yeast and Micr. lysodeicticus. The culture contained the virus only up to the 3d generation, which the authors explain by the survival of the virus. Since in control sowings in bouillon the virus was not detected in these generations, the authors think that in conditions of mixed culture the virus

finds conditions better for its preservation than in bouillon.

Entirely negative results were likewise obtained in the carefully set up experiments of Vinogradova (1936), Waldhecker (1936), and Portnlagina (1939).

All these materials permit our thinking that there were obtained no symbiotic cultures of the virus of rabies with the yeast. The positive results reported by certain authors are unconvincing and do not contain all the proofs needed of the specificity of the pathological processes caused by the cultures being investigated.

Offering far more interest are the works of Minervin's laboratory. Minervin studied the possibility of the carrying of the virus of rabies by microbes in natural conditions.

Minervin and Rapoport (1936) studied the microorganisms of the mouth cavity of rabid dogs in the character of bearers of the rabies virus.

The authors sowed the mucus of the oral cavity of dogs destroyed by rabies on dishes of blood agar and then after 2 days' growth transferred the isolated colonies to bouillon. The bouillon generations (up to the 7th) that had developed in the course of 3-5 days were tested by inoculation of rabbits, with subsequent passages of their brain when disease was present. The cultures were introduced into rabbits subdurally in very small dosage, from five to ten million microbe bodies. With the use of this method there was observed in the rabbits after 9-60 days of incubation a disease with the clinic characteristic to experimental rabies. The disease proved to be possible to pass several times from one rabbit to another, the organs and blood of the rabbits having been bacteriologically sterile. The authors did not observe in the brain of rabbits inoculated with cultures the typical Negri bodies, but saw formations similar in certain respects to these bodies. The clearest results were obtained with one gram-positive bacillus and with streptococcus.

These observations indisputably have great interest. Unfortunately they were not continued and set up sufficiently exten

sively. Meanwhile, namely in this direction, it is expedient also to study the problem of the symbiosis of the rabies virus with the microbes. At escaping from the central nervous system into the mouth cavity the virus of rabies is encountered in the saliva with many microorganisms, and it is necessary to seek among them the objects of possible symbiosis. It is necessary to take into consideration that this symbiosis is certainly not attended also by complete preservation of all the biological properties of the virus - it may be expressed simply in the fact that the virus in the microbe culture is preserved in those conditions of the medium in which by itself it dies. Unfortunately this question, a principal one from our point of view, has remained outside the field of vision of many investigators, namely from the need there would be to begin the study of the symbiosis of the rabies virus with the microbes.

As for other viruses that infect the central nervous system, then, inasmuch, as the majority of them penetrate into the nervous system, escaping into the nonsterile cavities and tissues of the animal organism, it is possible to judge their symbiosis with the microbes only in respect to those microbes with which these viruses are encountered in the organism of the conveyor. From this point of view it would be interesting to study the virus of tick encephalitis, inasmuch as it is transmitted by ticks from generation to generation and circulates in their organism for a long time. Of course, it is first necessary to clarify whether the virus circulates in the organism of the tick in nonsterile tissues.

In order to complete the account of data obtained during the study of the symbiosis of viruses that affect the nervous system with microbes, it is necessary to dwell briefly on data obtained with the virus of poliomyelitis and herpes.

Brutsaert and coworkers (1946) tried to cultivate the virus of mouse poliomyelitis in cultures of tick bacillus, a mixture of intestinal flora, of Leptospira, and of various protozoa. In cultures of Trichomonas hominis the virus may be found in the 4th generation, however close results were obtained with a killed culture of the same Trichomonas. It should be noted that the authors used media most favorable to the growth of the protozoa being studied, departing from our instructions on this problem.

In natural conditions obviously such media are lacking. Moreover, in a number of experiments they introduced into the animals filtrates of symbiotic cultures, evidently assuming that the virus is propagated in the medium and not in the microorganisms. Certainly these experiments could not give positive results. It is possible that they would not be positive too with observance of the necessary conditions of the experiment. Inasmuch as the herpes virus can be found with microbes of the integument, it would be possible to doubt in it the capacity to adapt itself to them.

Grundfest (1934 - translator's note: spelled Gruntfest in the bibliography) cultivated the virus of herpes with kephir yeast. The virus was observed in six subsequent generations, provoking typical affection and creating specific immunity in reinfected animals. In further generations the virus was not detected. Histological alterations in the brain of rabbits that died after inoculation with symbiotic cultures into the cornea were fully typical to the herpetic. Symbiotic cultures kept their virulence for a period of 3 months when preserved at room temperature.

Interesting data were obtained during the study of symbiosis with microbes of the virus of hoof and mouth disease. As early as 1933, together with E. I. Vostrukhova, we tried to obtain symbiotic cultures of the hoof and mouth disease virus. Experiments of obtaining these cultures by means of our strain of Torula kephir gave no positive results. More successful were investigations in which we tried to detect the carrier of the virus of hoof and mouth disease in natural conditions. At isolating from guinea pigs infected with hoof and mouth disease varibus cultures from their diseased paws, E. I. Vostrukhova separated out a sluggishly growing streptococcus, which indisputably was the carrier of the hoof and mouth disease virus and provoked in the pigs the typical disease. This property was preserved in it in eight resowings, whereas in control resowings of the virus on the same, however without a microbe medium, it was no longer detected in the third generation. These investigations were not continued and were not published.

Frenkel (1934) obtained negative results at endeavoring to obtain symbiotic cultures of the virus of hoof and mouth disease

with Torula kephir yeast. Poppe and Busch (1936) likewise obtained negative results in experiments with this yeast. However, the latter authors received indisputably positive results at cultivation of the virus of hoof and mouth disease in symbiotic cultures with Torula rubra and with certain other species of yeast. Cultures of the hoof and mouth disease virus with Torula rubra in the 60th generation provoked in guinea pigs not only a local but also a generalized process. The breeding of the hoof and mouth disease virus initially sown in the yeast culture reached the generation figure of $10^{62}$. With yeast of certain other species, Torula Kiel and L. (sic!), the experiments were also positive and the cultures contained virus in the 10th and 28th generations. With other species of yeast (Torula pulcherrima, the wine yeasts, and others) and with certain bacteria (staphylococcus, sarcina, and others) no symbiotic cultures were obtained.

The authors explained the importance of the presence of a neutral reaction of the medium (bouillon), in which the symbiotic culture is grown, as well as of the medium in which the virus is suspended at extraction of it from the tissue. For the latter purpose a phosphate buffer proved completely inadequate, whereas with Tyrode's solution good results were obtained. Thus, Poppe and Busch's experiments established with certainty the possibility of obtaining symbiotic cultures of the virus of hoof and mouth disease with certain species of yeast.

In symbiotic cultures the virus of the plague of swine was likewise studied. Likhachev (1937), proceeding from the fact that enterococcus and sarcinas are most often isolated from the blood of swine affected with plague, tried to cultivate the virus of this disease in symbiotic cultures with these microbes. The 5th, 10th, and 21st generations of these cultures produced at introduction into young pigs a pig plague typical in clinic and pathological-anatomical picture. The blood of the sick animal, tested in one case, produced the same disease at introduction into a healthy animal. For control there was an absence of virus in the installation in which the experiments were carried out, and there was a lack of contagiousness for the disease produced by the symbiotic cultures; in adjoining pens healthy young pigs were kept. These young pigs fell ill with plague on the 17th and 28th day after the start of the experiments and succumbed on the 21th

and 41st day, which indicates their infection from the experimental animals.

Cultures of enterococcus and sarcina without the virus produced no pathological phenomena in the young pigs. The animals that survived the introduction of the 21st generation of symbiotic culture were tested for immunity and did not disclose such. Earlier generations were not subjected to such testing. The experiments with two other strains of virus gave no positive results, but a total of three generations, the 20th, 5th, and 9th, were tested.

All the materials stated show that not only the virus of variolar vaccine can survive and multiply in conditions of symbiosis with certain microbes, but that this capacity is inherent to other viruses too.

## Experiments with Exanthematous Typhus

The provoker of exanthematous typhus is not, as known, an ultravirus (filtrable virus), but is a rickettsia. However, the rickettsias in the conditions of culture are quite similar to the viruses. They, as the latter, are obligatory cellular parasites and are incapable of multiplying on artificial nutritive media. These properties of theirs can make possible the adaptation of the rickettsias to other microbes, the more so that the provoker of exanthematous typhus is constantly found with microbes in the intestines of lice. The possibility of the alteration of certain properties of microbes under the effect of the virus of exanthematous typhus was demonstrated by us as early as 1922 when, at cultivation of Proteus vulgaris in the abdomen of a young pig with exanthematous typhus, a culture was obtained of the exanthematous typhus proteus, distinct from any whatsoever isolated from the exanthematous typhus organism. Minervin (1935), at introducing his exanthematous typhus into pig testicle, observed an analogous alteration of the Proteus vulgaris. It should be noted that the virus of exanthematous typhus possess no pronounced cytotropicity to cells of a definite species and type. Thus, it infects man, guinea pig, rabbit, and mice, and it multiplies in not only their brain but also in the tissue of the lungs, spleen and other tissues. All these data have also given us basis to

try to obtain symbiotic cultures of the exanthematous typhus virus. Experiments set up by approximately the same method as with the virus of the variolar vaccine (Zil'ber and Dosser, 1934) disclosed the presence of the exanthematous typhus virus in cultures with kephir yeast and sarcinas, in which the blood of exanthematous typhus (victims) was sown in a dosage of 0.2-0.3 ml. or 1/200 of the brain of exanthematous typhus pigs. The presence of the virus in these cultures was demonstrated first of all by experiments of inoculating pigs with them, the pigs having disclosed a temperature reaction typical to exanthematous typhus, the disease having been passed bacteriologically into sterile brain up to five times. In the diseased pigs rickettsias were discovered in the tunica vagia., as too the histopathological alterations characteristic to exanthematous typhus. In lice affected by symbiotic cultures by the rectal method rickettsias were discovered, and they died 15-20 days after inoculation. Inoculation of mice by the nasal route was not carried out since this method was not known at that time. Rabbits inoculated with symbiotic cultures produced an accumulation of agglutinins to Proteus $X_{19}$ in a titer of 1: 80, however not in all cases.

This work of ours attracted the attention of many investigators. Gel'tser and Hemshilov (1934) cultured the virus exanthematous typhus (the blood of patients) with Saccharomyces cerevisiae. The 15th, 16th, and 24th generations of these cultures were introduced into pigs, in which on the 12th-17th day after inoculation there was observed a rise of temperature within the limits of 0.8-2° and a duration of from 4 to 6 days. When passages were made with the blood and brain of these diseased pigs, the rise in temperature occurred on the 11th-18th day and the fever lasted 7-15 days (most often 8 days). In all the affected pigs there was noted a drop in weight. At introduction of cultures, as well as of brain of the passage pigs into rabbits there was observed in them the appearance of the Weil-Felix reaction with a titer of 1: 40 to 1: 60. In the brain of the affected pigs there were observed histopathologica alterations characteristic to exanthematous typhus. In a subsequent work Gel'tser and Hemshilov (1935) tested a further series of generations of symbiotic cultures, using for this purpose also kephir yeast, and obtained by this principally the same results.

In later generations of certain strains (18th-36th) the virus was not discovered, the weakening of the virus having proceeded with

different rapidity in different strains (strain I in the 34th generation, and strain III in the 18th generation). The experiments of the authors with the obtaining of immune sera by symbiotic cultures are quite substantial. These sera were obtained by immunization of horse, calf, and sheep and disclosed a considerable titer of the Weil-Felix reaction. Thus, the sera of horse and heifer raised the titer from 1: 100 to 1: 1200, and the sera of the sheep from 1: 50 to 1: 1200. These sera, tested after 42 days of preservation, in all experiments neutralized the passage virus of exanthematous typhus. Pigs into which a mixture of immune serum and virus was introduced gave no signs of exanthematous typhus and disclosed no immunity at their subsequent inoculation with passage virus. An attempt to vaccinate guinea pigs with symbiotic cultures killed in various ways produced no results, which is fully understandable in the light of contemporary data, since the number of virus in the symbiotic cultures was not sufficient for this purpose.

Iakovlev (1934) observed in cultures of the exanthematous typhus virus with yeast and sarcinas formations of various structure, which he considered to be different stages of the metamorphosis of the rickettsias. There have been offered no direct proofs of this.

Kalina and Danishevskaia (1933) in a preliminary report stated the positive results obtained at inoculation of the 24th and 49th generations of a yeast culture of the exanthematous typhus virus that had been transferred daily or every other day. However, the material cited was little conclusive since the typical temperature curve was observed in a total of one of the three pigs.

Symbiotic cultures of the exanthematous typhus virus up to the 6th and 8th generations were studied in detail by Tokarevich and Kliachko (1935). The disease typical to exanthematous typhus was observed in 13 out of 24 of the pigs inoculated with these cultures, the main indices characterizing the temperature curves having disclosed considerably greater diversity than is observed at inoculation of the pigs with passage virus. Histological alterations were discovered in four out of 12 of the pigs investigated, they having been discovered in three out of five cases with typical fever and in one out of seven cases with atypical fever. The cultures in a number of cases provoked the appearance of the Weil-Felix reaction in rabbits in a titer of 1: 40 and 1: 160. The immunity

was tested in a total of five pigs, one of them not having had a general fever at introduction of 0.1 of brain of passage pig and the other having given a brief (2-3 day) rise in temperature with major remissions. Levkovich (1934) also obtained principally the same results.

Afanas'eva and Sterkhova (1934) cultivated the European virus of exanthematous typhus in symbiosis with kephir and brewers' yeast. They came to the conclusion that such cultivation "succeeds in rare cases and in a limited number of generations", the exanthematous typhus virus proving slightly pathogenic and lessening its immunizing properties. Best results were obtained with yeast cultures of rat exanthematous typhus, which "disclose all the characteristic reactions natural to this virus and distinguish it from the epidemic, such as: the fever reaction in white mice, the fever and scrotal reaction in pigs, and the slight affection in them by granulomatosis of the cerebrum". However, all these indications in the cultures were expressed considerably more weakly than these were observed at affection by passage virus. The disease in pigs, provoked by yeast cultures of the virus, communicated immunity to the rat passage virus, but did not transmit immunity to the European. One of the cultures lost pathogenicity only in the 34th generation.

Elin, Frenkman (given as Frelman in the bibliography), and Urban (1935) tried to cultivate the virus of exanthematous typhus in symbiosis with yeast (with which of the yeasts is not indicated) At affection by some yeast cultures not containing the virus they observed in the pigs the temperature and histological alterations typical to exanthematous typhus. They also observed a positive Weil-Felix reaction in rabbits into which only the yeast was introduced.

The authors promised to report on further investigations of this paradoxical phenomenon, but this report did not follow.

Gnutenko and Friauf (1935) cultured the virus of exanthematous typhus in symbiosis with yellow sarcinas on a solid nutritive media. Out of the 12 experiments positive results were obtained in four cases.

Kompaneets and coworkers (1936) studied cultures of the virus of exanthematous typhus in symbiosis with yeasts (kephir, bread, and brewer's). The cultures were tested by inoculation of guinea pigs and by observation for the Weil-Felix reaction in rabbits and goats that had received these cultures.

The authors note the drop in virulency of the virus as beginning already in the second generation. However, rabbits which were immunized by the cultures, beginning with the 4th generation, gave in a considerable percentage of the cases a transition from the negative Weil-Felix reaction to the positive with a titer up to 1: 40, and in two the transition of the positive reaction was from a titer of 1: 60 to a titer of 1: 320. No virucidal properties were discovered in the goat serum.

Otto (1934) and Voet (1935) obtained negative results in a few attempts to cultivate the virus of exanthematous typhus in symbiotic cultures.

Minervin, Zil'berman, and Gerbil'skii (according to the bibliography, Gebril'skii) (1936) isolated from the intestines of lice taken from exanthematous typhus patients bacterial cultures which proved capable of producing in pigs at subcutaneous introduction typical exanthematous typhus fever and histopathological alterations entirely characteristic to exanthematous typhus. In certain cases the disease with a typical picture was passed by the brain of affected pigs bacteriologically sterile.

In a subsequent work Zil'berman and Gerbil'skii (1938) reported that the 4th and 6th generations of the virus cultures from bacteria isolated from lice did not disclose the presence of the virus at inoculation of pigs. Likewise, attempts to cultivate the exanthematous typhus virus in cultures of Proteus vulgaris, yeast, and sarcinas proved unsuccessful. Certain strains of Proteus vulgaris, formerly in contact with the blood of exanthematous typhus patients, acquired the ability to be agglutinated by the serum of rabbits immunized with $X_{19}$. Cultures of proteus, yeast, and sarcina that were taken into the experiment by the authors, by themselves without the virus, provoked in the pigs a number of pathological phenomena in a series of cases.

In judging the above-mentioned data it is necessary to take into consideration that all authors who have studied the properties of symbiotic cultures have compared them with a passage virus well adapted to the guinea pig organism. Of course, symbiotic cultures could not disclose in full measure those properties which are observed in a passage virus, but the presence of the exanthematous typhus virus in these cultures in many experiments was sufficiently demonstrated in a persuasive way. This was recognized by all these authors who carefully noted the absence of a complete conformity of the properties of the cultures and of the passage virus (Tokarevich and Kliachko, Gel'tser and Romshilov, Levkovich and others).

In certain cases the cultures of yeast that were taken for the experiments were insufficiently carefully selected and produced in the pigs certain pathological symptoms. However, in the majority of authors who studied this problem the cultures of the microbessymbionts were completely apathogenic to guinea pigs and produced no reaction in them, whereas the same cultures after contact with the exanthematous typhus virus produced in the pigs a number of symptoms typical to exanthematous typhus. The virus certainly weakens and modifies in symbiotic cultures, but, together with this, it is preserved in them in those conditions in which it dies when it is without microbes (for instance, during multi-month keeping in a thermostat).

Both in our experiments and in the experiments of other investigators rickettsias were not discovered with certainty in the symbiotic cultures, as the elementary bodies of the variola virus were not discovered. Apparently the rickettsias of exanthematous typhus can be represented either as formations of considerably lesser dimensions or as formations with a different than usual capacity for staining. By this apparently is explained too the difficulty of discovering the rickettsias in the brain of affected pigs. The problem of the symbiosis of the virus of exanthematous typhus with the microbes could be productively studied at the present time owing to the reproduction of pulmonary exanthematous typhus pneumonia in mice and the possibility of studying symbiotic cultures by the electron microscope.

General Resul s

The data cited above indicate sufficiently conclusively the existence of symbiosis between the microbes and viruses. The form of symbiosis described above was in due course designated as an "allobiophoria" (i.e. as a bearer of another life). Most often the term "virophoria", i.e. a carrier of viruses, is used. This last term is more concrete and better expresses the nature of the phenomenon. Entirely likely too is the existence of other forms of symbiosis besides the virophoria. In literature there are reports (Iakovlev, 1949, and others) in which it is indicated that the viruses survive in a medium populated with microbes, not entering into any closer connection with them. Evidently certain microbes can produce substances that make possible the survival of the viruses in the outer medium. This question has been very little studied.

The great similarity of the phenomena of bacteriophagy and virophoria attracts attention. Regardless of whichever point of view is adhered to in respect to the nature of the bacteriophage, it is impossible not to recognize that it possesses the principal properties that characterize the ultra-viruses - the capacity to propagate only in living cells, the lack of a capacity for growth on artificial nutritive media, the filtratability, the stability to certain disinfection substances, etc. Recently the data of the electron optics (?microscope) have permitted referring with certainty the bacteriophages to the ultraviruses. The processes observed in the presence of bacteriophagy are typically virophore phenomena. It is known that the phage is readily adsorbed by suitable bacteria and can in such an adsorbed form be preserved for a long time in bacterial cultures. If with this it was taken in comparatively small amount, then there occurs no dissolving in this culture, and it is a typically virophore culture. Those cultures of bacteria from which by different manipulations a bacteriophage is successfully obtained are such virophore cultures. It is interesting to note that at sowing the bacteriophage culture on agar the same phenomenon is observed as was noted at selection of virophore cultures of variola: not every colony is affected by phage and virus.

However, the bacteriophage is transferred ad infinitum

together with the bacterial cells, attaining vast concentrations and causing the dissolution of the cells, in contrast with the virus in the virophore culture, which, although it undergoes certain alterations, yet sooner or later is freed of virus, especially if it multiplies in favorable conditions. It is possible to suppose that the phenomenon of bacteriophagy in the process of evolution arose from the phenomenon of virophoria. The virus that had an affinity also to the animal cells and to the microbe (cells), and is probably nonpathogenic to the animal, can in the process of evolution acquire a more pronounced affinity for microbe cells and become pathogenic for them. We would not have resolved to express this hypothesis if we hadn't seen certain possibilities of its experimental verification. If we assume that permissibility the bacteriophage to the provoker of typhoid fever rises from the virophore culture prior to the latter microbe that bears the virus that had an affinity to the cells of the intestines, then it would be possible to expect that certain strains of this phage still preserve remnants of the affinity to the intestinal cells. It is possible to clarify this experimentally by studying the capacity of these cells to adsorb the phage and preserve it. Obviously for control there should be studied also the analogous capacity of the other cells. Such an investigation might also produce material that would permit using the phage more efficiently for purposes of prophylaxis and the treatment of infectious diseases.

In a further study of the phenomenon of virophoria it is impossible to break away from the theoretical foundation on which it is based. In virtue of this, first of all it is necessary:

1. To study the symbiosis of the viruses with those microbes with which a given virus is encountered in natural conditions. Hence, it is necessary to study the symbiosis of the virus of variola and of hoof and mouth disease with the microbes of the skin and mucous membranes of the corresponding animals, the virus of rabies with the microbes of the saliva of dogs, the virus of inguinal lymph-ogranulomatosis with the microbes of the skin, the virus of grippe with the microbes of the oral cavity and naso-pharynx, the virus of tick encephalitis with the microbes of the ticks, that of Japanese encephalitis with the microbes of mosquitoes, etc.

2. To study all forms of this symbiosis, and not only the

propagation of the virus in the symbiotic cultures. None the less, and more important in practice, is the phenomenon of the carrier proper, which is not obligatorily attended by multiplication of the virus in the microbe cell. Namely this virus carrier too should be studied in greatest detail.

3. To study virophoria necessarily first of all in natural conditions. By isolating the virophore bacterial cultures from diseased tissues it is easiest of all to obtain a clear idea both of the presence of symbiotic phenomena in a given infection and of their character. Of course, testing practices are an essential supplement to such experiments.

4. With this study substantial attention should be directed to the length of the maintaining of the virus in the virophore cultures and to the conditions of this preservation.

5. The study of the capacity of microbe cultures to become virophoric likewise should approximate natural conditions. For this purpose it would be necessary to introduce the culture being investigated into affected tissue and then to isolate it for several days.

Serious attention during the study of the virophore phenomena should be paid to those alterations which occur in the virophore cultures both in the microbe-carrier and in the virus. Meisel' (1935) observed a number of cytological alterations in yeast in symbiotic cultures of the virus of variola.

It should be noted that during all these investigations it is never possible for the determination of the presence of the virus to be limited only to clinical observations. It is always necessary to supplement them with the morphological (for instance, by the discovery of Paschen and Guarnieri bodies during variola) and by immunological investigations. This is necessary not only to determine the presence of the virus, but also to differentiate the phenomena provoked by it from those provoked by the microbe carrier, which likewise may be to one or another degree pathogenic. The simplest method for such differentiation is the inoculation by virophoric cultures both of healthy animals and of animals that have been treated, on the one hand, with serum for the virus and, on the

other hand, with serum for a given microbe. For instance, in studying the virophoric culture of the staphylococcus that bears the virus of the group, it is necessary to inoculate not only normal mice but also those which had received (the following therapy) prior to this introduction: one group - the anti-grippe serum, the other - the anti-staphylococcus serum. The results thus obtained permit making reliable conclusions even in a case in which the pathogenic microbe is studied in the character of carrier.

A study of the phenomena of the symbiosis of the ultramicrobes with the microbes does not have just theoretical interest. It is indisputable that in conditions of the symbiosis viruses are preserved longer than in the absence of microbes. Consequently, thanks to the virophoria, not only are the conditions of the dispersion of the viruses altered, but there is also formed a unique reservoir of viruses in nature. It is impossible to undervalue the importance of this supplementary reservoir for certain viruses. Earlier the impossibility of the existence in outer nature of ultraviruses pathogenic to man and animals was considered firmly established. It was thought that they exist only in the organism of these animals and in the organism of the vectors - the mosquitoes, ticks, lice, etc. Now it is shown that this is not so and that viruses, like phages, can exist in the outer medium in symbiosis with microbes. This circumstance should be taken into consideration in the epidemiology of certain ultravirus diseases, for which much is still in the dark up to the present time. It is enough even to recall the epidemiology of poliomyelitis. The phenomenon of the adsorption of the viruses by the microbes has practical importance too. Sergiev and coworkers (1945) and others have revealed that microbes that adsorb the viruses can be used in certain cases for discovery of antibodies to these viruses, the reaction of the agglutination of the microbes loaded with the virus possibly having diagnostic significance.

A study of the phenomena of the symbiosis of viruses and microbes gives substantial materials for understanding the inaccuracies of those investigators who, at isolating during virus diseases various microbes that are carriers of the viruses, have mistaken them for the provokers of those diseases. To the same category of phenomena belong also reports of the conversion of viruses into microbes.

It is sufficient to look at the microphotographs presented in Bosh'ian's book (1950) to be convinced that, for instance, the forms described by him as the spherical form of the provoker of infectious anemia of horses are yeast. Inasmuch as the yeast cannot be the provoker of this disease, it is possible to explain its specific infectiousness only by the fact that it is the carrier of the virus of infectious anemia.

As has been pointed out above, the phenomena of the symbiosis of viruses and microbes do not deplete by themselves those interrelations which exist in them in natural conditions. The presence in them of antagonistic relationships is definite too. The last question offers very great interest in connection with the discovery in recent years of the inhibiting effect of bacterial polysaccharides on the propagation of certain viruses. The further study of the problem of the interaction of the viruses and microbes, first posed and studied by Soviet scientists, will enrich science with facts of great theoretical and practical importance.

Literature

M. Akker and V. Florinskii. 1936. ZhMEI, 17 (3): 403.

A. Afanas'eva and N. Sterkhova. 1934. ZhMEI, 13 (3): 416, 424.

A. Afanas'eva and S. Shapiro. 1936. Tr. Mosk. gor. bakt. in-ta (1): 35.

G. Bosh'ian. 1950. On the Nature of Viruses and of Microbes.

A. Bulanov and V. Riakhovskii. 1936. ZhMEI, 16 (3): 394.

A. Vinogradova. 1936. ZhMEI, 17: 565.

R. Gel'tser and S. Nemshilov. 1934. Kaz. med. zhurn., 11-12: 1167. 1935 - ibid., 1: 86.

E. Gil'gut. 1934. ZhMEI, 14 (2).

M. Gmutenko and V. Friauf. 1935. Vestn. mikr., epid. i parazit., 14 (4): 325.

M. Gruntfest. 1934. ZhMEI, 12 (1): 62.

V. Elin, E. Frekman, and K. Urban. 1935. ZhMEI, 14 (3): 421.

L. Zil'ber. 1937. Tr. Vsesoiuzn. soveshch. po ul'tramikrobam i virusam (Transactions of the All-Union Conference on Ultramicrobes and Viruses), 219.

L. Zil'ber and A. Beliaeva. 1935. ZhMEI, 14 (3): 426.
L. Zil'ber and E. Vostroukhova. 1933, ZhMEI, 11 (1): 66.
1932. Sov. vrach. gaz., 15-16.
L. Zil'ber and E. Dosser. 1933. Sov. vrach. gaz., 15-16.
L. Zil'ber and V. Timakov. 1934. ZhMEI, 12 (3): 524.
B. Zil'berman and V. Gebril'skii. 1938. ZhMEI, 20 (5): 58.
M. Izabolinskii, I. Levtsov, and I. Cherniak. 1935. ZhMEI, 15 (1): 29.
G. Kalina and M. Danishevskaia. 1933. Sov. vrach. gaz., (12): 533.
A. Kompaneets, E. Maiofis, and A. Bocharova. 1936. ZhMEI, 17 (2): 282.
M. Korol'kova. 1935. ZhMEI, 15 (1): 33.
E. Levkovich. 1934. ZhMEI, 12 (1).
N. Likhachev. 1937. ZhMEI, 19 (2 (8)): 275.
M. Meisel'. 1937. Tr. Vsesoiuzn. soveshch. po ul'tramikrobam i virusam, 237.
S. Minervin, B. Zil'berman, and V. Gebril'skii. 1936. ZhMEI, 16 (3): 455.
S. Minervin and E. Rapoport. 1936. ZhMEI, 17 (3): 399.
G. Palavandov, A. Serebrianaia, and E. Pugach. 1936. Ann. Mechn. in-ta, 4 (2): 303.
L. Portniagina. 1939. ZhMEI, 9-10: 44.
P. Sergiev, N. Demina, and N. Riazantseva. 1945. ZhMEI, 3: 14.
P. Smirnov, Iu. Tutyshkina, N. Riasantseva, and Z. Stupina. 1935. ZhMEI, 15 (6): 852.
N. Solomin. 1942. Tr. Sverdl. in-ta mikr., 3(1): 80.
V. Timakov. 1936. ZhMEI, 16: 445.
K. Tokarevich and N. Kliachko. 1935. Sov. vrach. gaz., 23).
V. Tul'chinskaia. 1936. ZhMEI, 17 (3): 387 and 395.
E. Turevich and N. Ianushevich. 1937. Tr. Vsesoiuzn. soveshch. po ul'tramikrobam i virusam, 241.
L. Fal'kovich and N. Ianushevich. 1937. Tr. Vsesoiuzn. soveshch. po ul'tramikrobam i virusam, 257.
R. Khastovich and R. Shur. 1935. Ann. Mechn. in-ta, 1 (2): 377.
R. Khurgina. 1935. Giorn. Batter., 15: 946.
A. Iakovlev. 1934. Sov. vrach. gaz., (5). 1949, ZhMEI, 12: 28.
C. Amies. 1934. Brit. J. Exp. Path., 15: 185.

A. Arbeit. 1936. Ztrbl. f. Bakt., 134: 463.

P. Brutsaert, Cl. Jungeblut, and A. Knox. 1946. Proc. Soc. Exp. Biol. a. Med., 61: 265.

R. Dujarric de la Rivere. 1930. C. R. Soc. Biol., 104: 367.

C. Evans and K. Osterud. 1946. Science, 104: 51.

H. Frenkel, cited by Poppe and Busch.

C. Kling, G. Olin, J. Fahraeus, and G. Norlin. 1942. Acta Med. Scandin., 112: 217.

R. Otto. 1934. Dtsch. med. Wschr., 1299.

K. Poppe and G. Busch. 1936. Ztrbl. f. Bakt., I Abt., 136: 385.

J. Toomey, W. Takacs, and M. Schaeffer. 1948. Amer. J. Dis. Child., 75: 11.

J. Vost. 1935. C. R. Soc. Biol., 118: 951.

V. Young, O. Felsenfeld, and Byrd. 1949. Amer. J. of Clin. Path., 19: 1135.

M. Waldhecker. 1936. Ztrbl. f. Bakt., I.Abt., 135: 259.

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